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PRINCIPAL INVESTIGATOR: Alan B. Packard, Ph.D.

CONTRACTING ORGANIZATION: Children's Hospital Corporation
Boston, Massachusetts 02115

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Alan B. Packard, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Children's Hospital Corporation
Boston, Massachusetts 02115

E-Mail: alan.packard@tch.harvard.edu

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13. ABSTRACT (Maximum 200 Words)

The object of this project is the development of a PET radiopharmeaceutical for measuring multidrug resistance (mdr) in breast cancer. Multidrug resistance is resistance of a lesion to a specific class of drugs that includes many of the chemotherapeutics that are most effective against breast cancer. Single-photon myocardial perfusion agents such as ^{99m}Tc-MIBI are substrates for Pgp, the protein implicated in mdr, and are now being studied for evaluation of mdr. A PET mdr tracer would provide significant advantages over ^{99m}Tc-MIBI, and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the mdr process in breast cancer (~240 min.) than are other PET radionuclides (e.g., ^{11}C, $T^{1/2}$= 11 min). We are carrying out in vivo and in vitro studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals as potential PET mdr radiopharmaceuticals using murine (MAT-B) and human (MCF-7) breast cancer models. In vitro studies of prototype ^{64}Cu-based complexes reveal a pattern of uptake similar to that observed for ^{99m}Tc-MIBI. Studies are currently underway to determine that optimal chemical properties of this agent. The development of a radiopharmaceutical for the measurement of the mdr status of breast cancer lesions will facilitate optimization of treatment protocols, monitoring of the development of acquired resistance, and real-time evaluation of mdr modulators.

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Breast Cancer, PET, ^{64}Cu, Multidrug Resistance

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INTRODUCTION

The objective of this project is the development of a PET (Positron Emission Tomography) radiopharmaceutical for the quantitative functional evaluation of multidrug resistance (MDR) in breast cancer. Multidrug resistance (MDR) is defined as intrinsic or acquired resistance to a specific class of chemotherapeutic drugs, which includes many of the most effective chemotherapeutic agents against breast cancer.

Multidrug resistance is characterized by overexpression of the *MDR1* and *MRP* genes and increased concentrations of P-glycoprotein (Pgp), a 170 kD transmembrane glycoprotein, and multidrug-resistance protein (MRP1), a 190 kD protein, which reduce the intracellular concentration of the drugs to non-toxic levels. Lipophilic cationic complexes such as ^{99m}Tc-MIBI are substrates for Pgp and MRP1 and are now being studied for clinical evaluation of MDR. We are carrying out *in vivo* and *in vitro* studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals derived from copper-diiminedioxime (PreH) complexes (Fig. 1) as possible PET MDR radiopharmaceuticals.

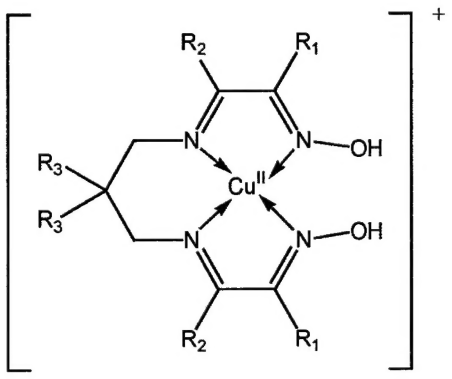


Fig. 1. Copper(II) Diiminedioxime (PreH) Complex

A PET MDR tracer will provide significant advantages over ^{99m}Tc-MIBI (e.g., straightforward attenuation corrections, higher spatial resolution, greater sensitivity, and the ability to perform quantitative studies), and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the MDR process (~240 min.) {1} than are other PET radionuclides such as ^{11}C ($T_{1/2}$= 11 min). Furthermore, ^{11}C-based radiopharmaceuticals are only available at a limited number of institutions and, because of the short half-life of ^{11}C, cannot be shipped to other institutions. This new radiopharmaceutical will provide real-time information about the MDR status of breast cancer lesions that may allow optimization of treatment protocols, monitoring of the development of acquired resistance, and evaluation of the effectiveness of drugs developed to modulate MDR.

BODY

The research accomplishments are discussed in terms of each Task outlined in the Revised Statement of Work (6/1/99).

Task 1: Recruitment and training of research technician, Months 1-3

Ms. Erica A. Guice was recruited in October 1999 and resigned in December, 2000. Immediately prior to and after her departure an extensive effort was made to recruit a new technician to fill this position but no acceptable candidates were identified until spring 2001. The absence of acceptable candidates during this period was presumably due to the extremely low unemployment rate in the Boston area at that time and the paucity of recent science graduates looking for technical positions. In mid-spring 2001 approximately 50 applications were reviewed, and interviews were conducted with six finalists. Following these interviews, Mr. Robert Borgesi, who received his B.S. degree in Biology from Boston College in May, 2001, was hired to fill this position effective June 4, 2001. After approximately one month of training and new employee orientation, we made rapid progress in the establishment and validation of the breast cancer cell lines. Mr. Borgesi resigned from the Research Technician position in November, 2002. Because there was less than one year remaining in the project, it was not possible at this point to fill the Research Technician position on other than a temporary basis.

On February 1, 2003, Carmen Solorzano, Ph.D. joined the project but resigned on May 15, 2003, to accept a postdoctoral position. The position has been vacant since that time, and Dr. Packard has, through necessity, carried out as many of these responsibilities as possible.

Task 1 has been completed.

Task 2: Establish and validate *in vitro* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines
b. Establish MDR breast cancer cell lines
c. Validate parental and MDR breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and MDR breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

Two breast cancer cell lines have been established, the rat MAT-B cell line and the human MCF-7 cell line. The MAT-B cell line is an ascites tumor derived from the rat 13762 solid mammary adenocarcinoma. The multidrug-resistant MAT-B cell line, MAT-B/r was established in the laboratory of our collaborator, Dr. Alun Jones, at Harvard Medical School, and is now maintained in our laboratory. This cell line has the advantage that is native to the Sprague-Dawley rat and is, therefore, expected to show more physiologically relevant perfusion in *in vivo* studies.

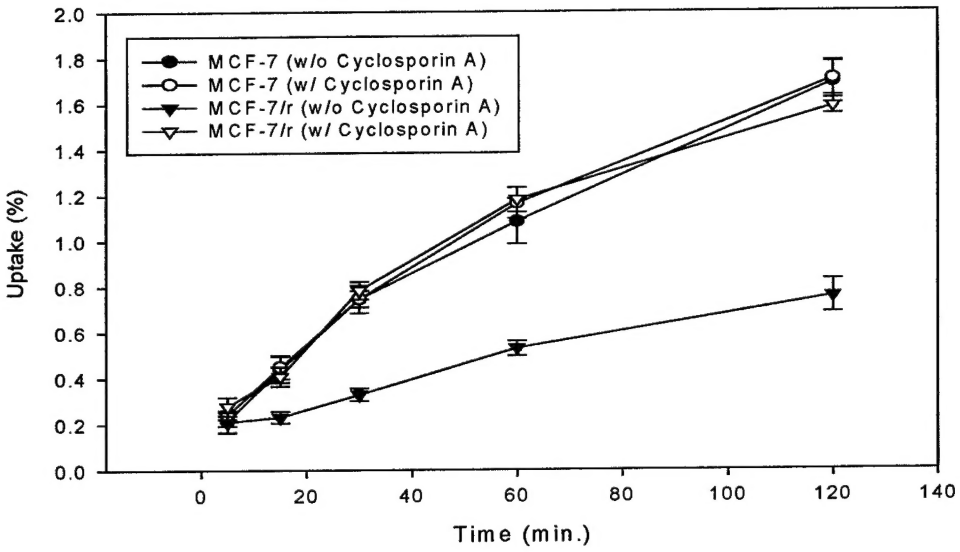
The MCF-7 is a human mammary adenocarcinoma cell line. This cell line is more relevant to the problem of human breast cancer, but *in vivo* studies must be carried out as xenografts in nude mice with the associated differences between the two species. This cell line and its MDR variant were obtained from Robert Gillies at the University of Arizona and the Arizona Cancer Center and are currently being maintained in our laboratory.

Both the MAT-B and MCF-7 parental and drug resistant cell lines were validated *in vitro* with ^{99m}Tc-MIBI. These studies confirmed both the lower uptake of ^{99m}Tc-MIBI by the drug-resistant cells and the ability of Cyclosporin A, a well-known MDR reversal agent, to counteract drug resistance in these models.

In vitro studies were carried out with the MAT-B and MCF-7 (resistant and parental) cell lines and the ^{64}Cu PreH complex ($[^{64}Cu(Me_2MAKPreH)]^+$) ($R_1 = -(CH_2)_3CH_3$, $R_2 = R_3 = -CH_3$). In summary, the parental and resistant MCF-7 cells were equilibrated with $[^{64}Cu(Me_2MAKPreH)]^+$ at 37° C, samples were removed at selected time intervals, and the uptake of ^{64}Cu by the cells was measured. The experiment was also carried out using media containing 10 μM Cyclosporin A, a known MDR reversal agent.

The results of a typical study with the parental and drug-resistant MCF-7 cell lines are shown graphically in the Figure 2. As can be seen this graph, the uptake of the ^{64}Cu complex by the resistant cell line (-▼-) is low relative to that of the parental cell line (-●-). In the presence of Cyclosporin A, however, there is no difference between the uptake of the ^{64}Cu complex by the parental (-○-) and resistant (-▽-) cell lines. This result supports the hypothesis that the uptake of these cells is lower in drug-resistant breast cancer cells than in non-resistant cells, which in turn suggests that these complexes may be useful for PET evaluation of multidrug resistance in breast cancer. Additional studies will examine the mechanism of uptake as well as evaluate the relationship between the chemical biological properties of the copper complexes.

Figure 2. Uptake of $^{64}Cu(Me_2MAKPreH)$ by MCF-7 Cells



This study was also carried out using the MAT-B cell lines, and a similar result was obtained. No studies have yet been carried out with the ^{64}Cu-"cyclops" (cyclops = BF_2-closed macrocyclic derivative of PreH) derivatives. These complexes are expected to be more stable *in vivo* than the hydrogen-bonded, pseudomacrocyclic PreH derivatives, but they are somewhat more difficult to prepare at the no-carrier-added level.

Task 2 has been completed.

Task 3: Establish and validate *in vivo* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines in animal model
b. Establish MDR breast cancer cell lines in animal model
c. Validate parental and MDR breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and MDR breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

The parental and resistant MAT-B and MCF-7 breast cancer cell lines were established.

The MAT-B cell line was established in the Sprague-Dawley rat after an amendment to the animal protocol was submitted and approved that allows us to carry out studies using the parental and drug-resistant MAT-B cell lines simultaneously in the same animal. No *in vivo* studies have been undertaken to date with the MCF-7 cell line because at this point the additional information that may be gained by using the human cell line is not considered to offset the significantly higher cost of the nude mice required for these studies.

An *in vivo* study using the MAT-B tumor model and the prototype ^{64}Cu complex, $[^{64}Cu(Me_2MAKPreH)]^+$, was carried out in Sprague-Dawley rats. The non-resistant tumor was implanted in the right thigh (10^6 cells) and the drug resistant tumor was implanted in the left thigh (10^6 cells). The tumors were allowed to grow for approximately two weeks at which point the biodistribution study was carried out. Each animal was injected with 25 μCi $[^{64}Cu(Me_2MAKPreH)]^+$ and sacrificed at selected time intervals post injection. The results of this study are summarized below (Table 1).

Table 1. Biodistribution of [64Cu(Me2MAKPreH)]+ (% i.d./g, n=5)

Tissue	Time Post-Injection (min.)		
	5	15	60
Blood	0.41 ± 0.05	0.29 ± 0.06	0.36 ± 0.02
Heart	1.55 ± 0.11	1.10 ± 0.14	0.92 ± 0.11
Lungs	1.45 ± 0.11	0.75 ± 0.14	0.54 ± 0.04
Liver (sample)	6.26 ± 0.76	5.26 ± 0.98	1.55 ± 0.23
Spleen	2.15 ± 0.40	1.64 ± 0.37	0.56 ± 0.07
Kidneys	10.26 ± 1.37	8.48 ± 1.67	3.90 ± 0.75
Gut	0.52 ± 0.16	0.52 ± 0.25	0.79 ± 0.49
Brain	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Skin/Fur/Fat	0.22 ± 0.06	0.28 ± 0.06	0.31 ± 0.04
Muscle	0.23 ± 0.03	0.24 ± 0.04	0.28 ± 0.05
Bone	0.40 ± 0.05	0.37 ± 0.08	0.32 ± 0.06
Tumor (resistant)	0.21 ± 0.04	0.21 ± 0.05	0.46 ± 0.19
Tumor (non-resistant)	0.30 ± 0.04	0.30 ± 0.06	0.38 ± 0.08

The most interesting observation from the point of view of this project is that at 5 and 15 min. post-injection, the uptake of ^{64}Cu is lower in the resistant than in the non-resistant tumors ($P<0.05$). This is the result that would be expected if the ^{64}Cu complex is a substrate for Pgp. There is, however, no difference between the resistant and non-resistant tumors at the 60 min. time point ($P>0.10$). The pattern of uptake is similar to that observed with ^{99m}Tc-MIBI except for the increase in uptake by the resistant lesion at 60 min. post-injection, which is marginally significant ($P=0.05$).

It is also interesting to note that the amount of tracer found in the heart in this study is lower than was observed in previous studies with this complex (3.0-3.5% i.d./g). The earlier studies were, however, carried out with low-specific-activity ^{64}Cu, which raises the possibility that there may be some decomposition of the complex *in vivo*. Prior to undertaking these studies, however, we will repeat the biodistribution study to determine if this result is real or anomalous, perhaps because of decomposition of the complex prior to injection or injection of the wrong compound. If the second result replicates the first, we will carry out several experiments to ascertain the reason(s) for the difference between the low specific-activity and high specific activity results.

First, we have already repeated the *in vitro* studies with this compound to determine if addition of the non-radioactive complex, which may inhibit decomposition, increases uptake in the MAT-B cells. This study was carried out using 3 different concentrations of Cu^{II} ranging from no-carrier-added (>100 mCi/μg, <1 μM) to 1 mg/mL (1.57 mM). There was no difference in either the total uptake of the tracer by the tumor cells or in the differentiation between resistant and non-resistant cells, which is consistent with our previous observations that these complexes are stable *in vitro*.

Second, we will repeat the biodistribution experiment using different concentrations of non-radioactive Cu^{II}, similar to the *in vitro* experiment described above, to ascertain the role, if any, of non-radioactive Cu^{II} on the biodistribution. This experiment will also include a single animal that will be injected with a larger amount of tracer. A blood sample will be obtained from this animal and analyzed to determine the chemical form of the tracer that is present in the circulation.

Third, we have developed a rapid, high-yield synthesis for the "closed" (cyclops) complex in which the hydrogen bond between the two oxime moieties (Fig. 1) is replaced by a covalent BF_2 linkage. This complex is expected to be more stable *in vivo* because it is a "real" macrocycle

(i.e., one closed by a covalent bond) as opposed to the pseudo-macrocycle (i.e., one closed by a hydrogen bond) in the PreH complex. Both *in vitro* and *in vivo* studies will be carried out with this compound to determine if the type of ring closure affects the biological properties.

Additional *in vivo* studies will be carried out as we identify, based on the *in vitro* studies, ^{64}Cu complexes that have higher cell uptake or greater resistant/parental differentiation than the prototype complex, $[Cu(Me_2MAKPreH)]^+$.

An additional aspect of the validation of the MAT-B and MCF-7 cells lines is measurement of Pgp in the parental and resistant lines. This measurement is carried out by Western Blot assay using the C219 antibody {2}. We have recently acquired a Bio-Rad Criterion electrophoresis apparatus and power supply as well as a Criterion blotting apparatus (for Western Blots). This will allow us to carry out these assays in house where we previously had to carry them out at Harvard Medical School.

Prior to acquiring our own apparatus, we carried out a Western Blot assay for Pgp on the parental and drug-resistant MES-SA cell lines. These cell lines were chosen as a starting point because their Pgp expression is well-known. The assay confirmed that the Pgp concentration in the resistant sub-line was higher than in the parental line.

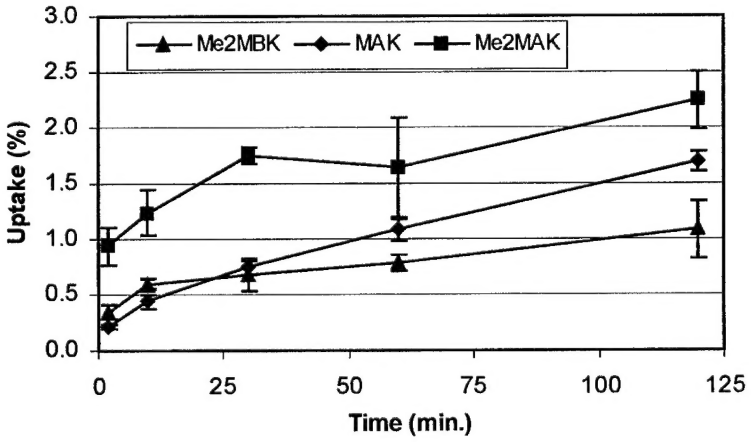
We have also recently acquired an inverted microscope and other related apparatus that will facilitate cell studies within our own institution where we previously were carrying these out partially at Children's Hospital and partially at Harvard Medical School.

Parts a and b of this task have been completed. The acquisition of the electrophoresis apparatus described above will allow us to continue to pursue Parts c and d after the expiration of the current project.

Task 4: Evaluate the effect of chemical properties (lipophilicity, ligand geometry, ligand substituents) on the biological properties of a copper-based PET radiopharmaceutical for the functional assessment of multidrug resistance in breast cancer, Months 13-24
- a. *In vitro* studies of the ^{64}Cu-labeled complexes using breast cancer model
- b. *In vivo* studies of the ^{64}Cu-labeled complexes using breast cancer model

In vitro studies were carried out on two ^{64}Cu diiminedioxime complexes that are chemically similar to $[Cu(Me_2MAKPreH)]^+$. $[Cu(Me_2MAKPreH)]^+$ was chosen as the starting point because its uptake was clearly superior to that of less lipophilic complexes with the MES-SA cell line.

Figure 3. Comparison of Uptake of Several Diiminedioxime Derivatives in MCF-7 Cells.



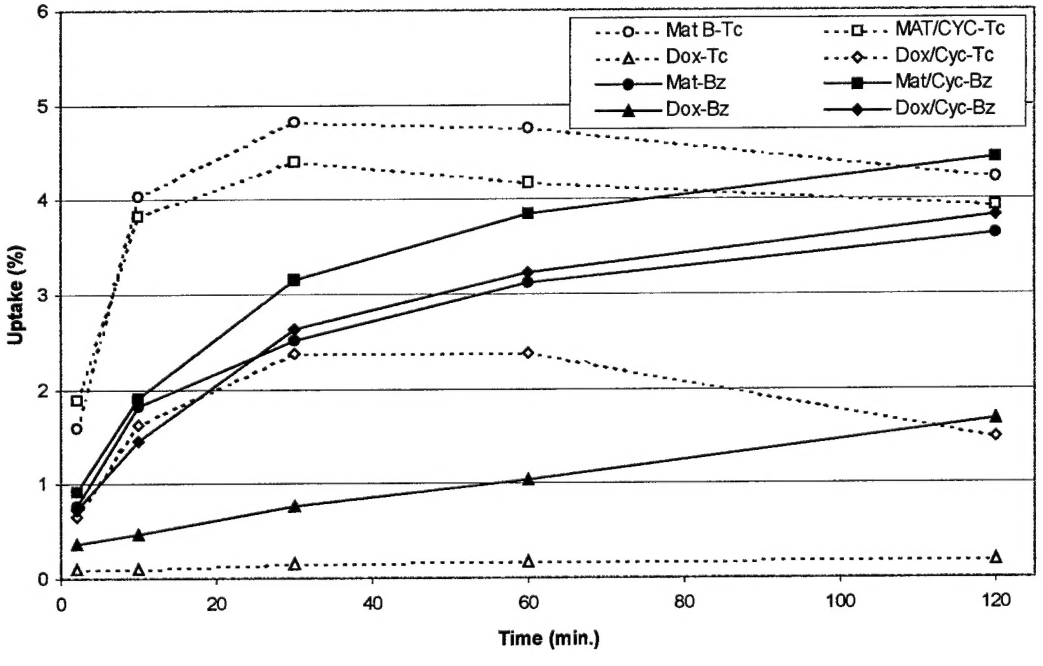
The two ligands were Me_2MBKPreH (R_1 = $(CH_2)_2CH_3$, R_2 = CH_3, R_3 = CH_3) and MAKPreH (R_1 = $(CH_2)_3CH_3$, R_2 = CH_3, R_3 = H), which each have two fewer carbon atoms than Me_2MAKPreH. These studies were carried out using the MCF-7 cell line. For clarity, only the results for the parental line without Cyclosporin A are shown in Figure 3.

As can be seen from this graph, the uptake of the complexes by the MCF-7 cells is, as expected, lower for both compounds, as predicted for complexes that are less lipophilic than $[Cu(Me_2MAKPreH)]^+$. There is perhaps a small difference between the two less lipophilic complexes, which have similar lipophilicity but different geometry, at 120 min. Other results (not shown) for the resistant cells and the effect of Cyclosporin A follow the pattern previously observed for $[Cu(Me_2MAKPreH)]^+$ in other cell lines. This experiment will be repeated with with complexes that have the same number of carbon atoms as $[Cu(Me_2MAKPreH)]^+$ but different geometry (e.g., $[Cu(MHKPreH)]^+$, (R_1 = $(CH_2)_4CH_3$, R_2 = CH_3, R_3 = H), and with complexes that contain two more carbon atoms than $[Cu(Me_2MAKPreH)]^+$ (e.g., $[Cu(Me_2MHKPreH)]^+$, (R_1 = $(CH_2)_4CH_3$, R_2 = R_3 = CH_3) to further evaluate the effects of ligand geometry, if any. It will also be repeated using the MAT-B cell lines to determine if the pattern of response is similar between the two cell lines.

The current focus of our investigation is the introduction of methoxy or ethoxy substituents into the ligand. Previous studies of other radiopharmaceuticals that are also substrates for Pgp have shown that in all cases inclusion of these functional groups improves the biological properties of the complex. Our original effort was directed towards the use of alkyl ether substituents at the R_1 position, but we encountered significant problems in the synthesis of ligands including these moieties. To circumvent this problem, we are now preparing derivatives that include aryl ethers at the R_1 position (e.g., 4-methoxybenzyl). As the first step in this direction, we prepared the benzyl derivative, $[^{64}Cu(benzylPreH)]^+$ (R_1 = $-CH_2-C_6H_5$, R_2 = CH_3, R_3 = H) and measured its uptake in the MAT-B cell line. The results of this study are summarized in Figure 4, which compares the cell uptake of the benzyl complex to that of ^{99m}Tc-MIBI.

In this graph, the ^{99m}Tc-MIBI data is shown with dotted lines, and the ^{64}Cu-benzylPreH data is shown with solid lines. The parental MAT-B data are shown with circles (● - ^{64}Cu, ○ - ^{99m}Tc), the parental MAT-B data with Cyclosporin A are shown with squares (■ - ^{64}Cu, □ - ^{99m}Tc), the resistant MAT-B data without Cyclosporin A are shown with triangles (▼ - ^{64}Cu, ▽ - ^{99m}Tc, and the resistant MAT-B data with Cyclosporin A are shown with diamonds (♦ - ^{64}Cu, ◊ - ^{99m}Tc).

Figure 4. Uptake of ^{99m}Tc-MIBI and ^{64}Cu-BenzylPreH by MAT-B Cells.



These data show that the uptake of this PreH derivative is 65% that of ^{99m}Tc-MIBI at 60 min (3.1% vs. 4.7%) and more than 85% that of ^{99m}Tc-MIBI at 120 min. (3.6% vs. 4.2%) This result is the closest to ^{99m}Tc-MIBI that we have achieved to date. In comparison to the prototype ^{64}Cu complex, the cell uptake of the benzyl complex is more than twice that of $[^{64}Cu(Me_2MAK)]^+$ at both 60 (3.1% vs. 1.3%) and 120 min. (3.6% vs. 1.6%). At all time points, it shows the expected pattern of uptake by the resistant and non-resistant cells as well as the expected response to Cyclosporin A. This result is especially encouraging because the study of the benzyl complex was only carried out as a baseline for comparison with the 4-methoxybenzyl derivative.

We recently completed the synthesis of the 4-methoxybenzyl derivative, including synthesis of the no-carrier-added ^{64}Cu complex, and carried out an *in vitro* study using with this complex and the MAT-B cell line. Interestingly, this complex showed total cell uptake similar to that of the benzyl derivative but no apparent differentiation between the parental and resistant cell lines and no effect on the addition of Cyclosporin A. This suggests that the complex may be simply sticking to the cells through a lipophilic interaction even though we have not observed this previously. Further studies are underway to better understand this result.

We have also initiated studies aimed at determining the mechanism of uptake of the ^{64}Cu complexes by breast cancer cells. These cells are patterned after similar studies with ^{99m}Tc-MIBI in chick myocytes and fibroblasts {3, 4}. In summary, the uptake of the ^{64}Cu complexes by tumor cells will be measured in the presence and absence of various metabolic inhibitors. These compounds include valinomycin, which depolarizes the mitochondrial membrane, nigericin, which hyperpolarizes the mitochondrial membrane, and high K buffer, which depolarizes the cell membrane. We had initiated these studies last year but were forced to discontinue them when Mr. Borgesi resigned.

We will continue to test only the most promising complexes *in vivo*. As previously described, these studies will use two separate animal models. The primary model will be the MAT-B (rat) model which was used in the biodistribution study of $[Cu(Me_2MAKPreH)]^+$ described above. This model will be used for standard biodistribution studies. These have been initiated and will continue as promising new complexes are identified in the *in vitro* studies. Additional studies will be carried out using nude mice bearing MCF-7 tumors as indicated by the results of the studies with the MAT-B model.

Both parts a and b of this Task will continue after the expiration of the current project.

Task 5: Evaluate differences between biological properties of the ^{64}Cu PET radiopharmaceuticals in breast and non-breast MDR tumor models, Months 13-24

The uptake of $[^{64}Cu(Me_2MAKPreH)]^+$ in the parental and resistant breast cancer cell lines, MCF-7 and MAT-B, was compared to the uptake in the parental and resistant MES-SA cell lines. This comparison revealed a similar pattern for all three types of cells. In each case, a maximum uptake of approximately 2% was observed for the parental cells after 2 hours. This compares to 0.2% - 1.2% for the resistant lines. For all three cell lines, addition of Cyclosporin A significantly increased uptake of the tracer by the cells.

This Task has been completed for the $[^{64}Cu(Me_2MAKPreH)]^+$. It will be repeated as necessary as promising complexes are identified and will continue after the expiration of the current project.

Task 6: Integrate results of Task 4 and Task 5 into the development of a ^{64}Cu-based PET radiopharmaceutical for the evaluation of MDR in breast cancer, Months 13-36.

This Task has continued throughout the project and will continue after the expiration of the project.

KEY RESEARCH ACCOMPLISHMENTS

- *In vivo* validation of parental and drug-resistant MAT-B (rat) breast cancer cell lines with prototype ^{64}Cu-Me$_2$MAKPreH complex
- Western Blot assay of Pgp in parental and resistant cells validated with MES-SA cells
- *In vitro* comparison of effect of variation in substituent position on ligand on uptake by parental and resistant MCF-7 cells.
- *In vitro* studies of benzyl diiminedioxime derivative that show cell uptake approaching that observed for ^{99m}Tc-MIBI.
- Preliminary studies of mechanism of uptake using metabolic promoters/inhibitors in breast cancer cell lines
- Comparison of uptake of [^{64}Cu(Me$_2$MAKPreH)]$^+$ between the MAT-B and MCF-7 breast cancer cell lines and MES-SA cell lines

REPORTABLE OUTCOMES

Abstracts and presentations (Copies are included in the Appendix)

1. Packard AB, Kiani S, Guice E. "Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance." PacifiChem 2000, Honolulu, HI, December, 2000, #MEDI-66.
2. Kiani S, Packard AB. "Radiosynthesis and *in vitro* study of ^{64}Cu-labeled diiminodioxime complexes as putative PET imaging agents for evaluation of multidrug resistance (MDR).", 12th International Radiopharmacology Symposium, Interlaken Switzerland, June, 2001
3. Kiani S, Packard AB. "The development and in vitro characterization of lipophilic cationic copper(II) complexes as potential PET radiopharmaceuticals for the functional evaluation of multidrug resistance.", NERM 2001, Durham, NH, June, 2001
4. Packard, AB, Kiani S, Borgesi R, Barbarics E. "Development of a ^{64}Cu-based PET Radiopharmaceutical for imaging MDR." Era of Hope, Orlando, FL, September, 2002.

Cell lines

The MES-SA and MES-SA/Dx5 (human uterine sarcoma) cell lines are established and being used for method validation.

The parental and multidrug-resistant MAT-B (rat mammary adenocarcinoma) cell lines are established and validated.

The parental and multidrug-resistant MCF-7 (human mammary adenocarcinoma) cell lines are established and validated.

CONCLUSIONS

We are now using the MAT-B (rat) and MCF-7 (human) breast cancer cell lines *in vivo* and *in vitro* to evaluate new ^{64}Cu complexes. We have also undertaken preliminary studies to investigate the mechanism of uptake of these compounds. As part of our effort to prepare an ether-functionalized PreH derivative, we prepared a benzyl derivative which shows 2-3 times higher cell uptake than the prototype ^{64}Cu complex, [Cu(Me$_2$MAKPreH)]$^+$. We also compared the uptake of [Cu(Me$_2$MAKPreH)]$^+$ in three cell lines, including the MES-SA (non-breast), and observed that the total accumulation, the pattern of uptake in the resistant and parental cells,

and the response to Cyclosporin A is similar for all three. We also observed that there is a difference in uptake between resistant and non-resistant MAT-B tumors *in vivo*.

Although these results are preliminary, they demonstrate that the pattern of uptake of this complex parallels the drug resistant status of the tumor cells, which supports our hypothesis that these complexes may prove useful as PET radiopharmaceuticals for the evaluation of MDR in breast cancer. Additional studies are carried out in parallel with the biweekly deliveries of ^{64}Cu. These results are being added to a database that will be used to develop structure/biodistribution relationships that will guide the development of new copper complexes.

Although BCRP support for this project will expire 8/31/03, we will continue to evaluate this class of compounds as potential PET radiopharmaceuticals for evaluating multidrug resistance in breast cancer as outlined above.

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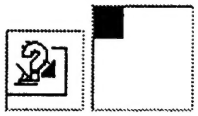
3. Chiu ML, Kronauge JF, Piwnica WD. Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium(I) in cultured mouse fibroblasts. J Nucl Med 1990;31:1646-53.
4. Piwnica-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence. Circulation 1990;82:1826-38.

APPENDIX

Abstracts

1. Packard AB, Kiani S, Guice E. "Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance." PacifiChem 2000, Honolulu, HI, December, 2000, #MEDI-66.
2. Kiani S, Packard AB. "Radiosynthesis and *in vitro* study of ^{64}Cu-labeled diiminodioxime complexes as putative PET imaging agents for evaluation of multidrug resistance (MDR).", 12th International Radiopharmacology Symposium, Interlaken Switzerland, June, 2001
3. Kiani S, Packard AB. "The development and in vitro characterization of lipophilic cationic copper(II) complexes as potential PET radiopharmaceuticals for the functional evaluation of multidrug resistance.", NERM 2001, Durham, NH, June, 2001
4. Packard, AB, Kiani S, Borgesi R, Barbarics E. "Development of a ^{64}Cu-based PET Radiopharmaceutical for imaging MDR." Era of Hope, Orlando, FL, September, 2002.



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Abstract

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Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance.

A. Packard*, S. Kiani, E. Guice, E. Guice, *Harvard Medical School, Nuclear Medicine, 300 Longwood Avenue, Boston, MA, 02115, USA, Fax: 617-232-0517

The aim of this work is to develop ^{64}Cu-based PET radiopharmaceuticals for the functional evaluation of multidrug resistance in breast cancer and other malignancies. We previously identified the lipophilic cationic copper(II) complexes of the diiminedioxime ligands as promising candidates for this application using low specific-activity ^{64}Cu (2 mCi/mg) and carried out preliminary biological studies that confirm the potential utility of these complexes for this application. We have now developed a rapid synthesis (<5 min.) for use with high specific-activity (HSA) ^{64}Cu (>10^5 mCi/mg) that produces these complexes in 90% yield and 90% radiochemical purity and characterized the products by HPLC and ITLC using the "cold" complexes as standards. In vitro stability was tested by equilibration of the ^{64}Cu-complex with 2.5% BSA/PBS using a Sephadex G-50 column, which revealed no evidence of plasma binding or transchelation of ^{64}Cu to albumin. Additional in vivo and in vitro studies are currently underway.

ABSTRACT FORM: 12th International Symposium of Radiopharmacology, June 12-15, 2001 - Interlaken (Switzerland)

TITLE IN CAPITAL LETTERS
Authors' names, preceded by initials in lower cases; underline presenting authors' name. Institution name in lower cases.

RADIOSYNTHESIS AND IN VITRO STUDY OF ^{64}CU-LABELED DIIMINODIOXIME COMPLEXES AS PUTATIVE PET IMAGING AGENTS FOR EVALUATION OF MULTIDRUG RESISTANCE (MDR)

S. Kiani, A. Packard, Children's Hospital, Harvard Medical School, Boston, MA, USA

The aim of this study is to develop a copper-based PET compound for the evaluation of multidrug resistance (mdr) in cancer. For this purpose, we investigated the effect of variations in the alkyl substituents on the cellular accumulation of a series of ^{64}Cu complexes of pseudomacrocyclic diiminedioxime ligands. The uptake of the ^{64}Cu complexes was measured in vitro in the MES-SA uterine sarcoma cell line and its multidrug-resistant DX5 derivative. The effect of the mdr reversal agent Cyclosporin A on the uptake was also measured. The stability was tested by equilibration of the ^{64}Cu-complex with human serum/PBS, which revealed no evidence of plasma binding or transchelation of ^{64}Cu to serum proteins. A systematic increase in cell uptake was observed as the length of the alkyl substituent (and thus the lipophilicity) increased from methyl (log P = -2) through pentyl (log P = 2). There was a corresponding increase in the difference between the uptake by resistant and non-resistant cells. These results suggest that ^{64}Cu diiminedioxime complexes are promising agents for PET evaluation of MDR.

Text should contain: a) aim of the study; b) methods; c) summary of the results; d) discussion and conclusion. Do not include references. Type the text including any tables in single line spacing. Take care not to type outside the blueline. Use standard abbreviations. Place unusual abbreviations in parentheses after the full word the first time it appears. Clearly identify all radio-pharmaceuticals used; standard abbreviations may be used (MDP, HMPAO, MIBI, etc.). For good reproduction the typing should be sharp and regular, using courier type 12. Please do not use smaller type because the abstract will be reduced to 70% during the reproduction process.

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Abstract title Radiosynthesis and in vitro study of ^{64}Cu-labeled diiminedioxime complexes as putative PET imaging agents for mdr

Name of presenting author Alan B. Packard, Ph.D
Institution Children's Hospital/Harvard Medical School
Department/University/Hospital Division of Nuclear Medicine, Children's Hospital Harvard Medical School
Full address 300 Longwood Ave
Postal code, City and Country Boston, MA, USA 02115
Telephone (617) 355-7579 Telefax (617) 232-0517
E-mail alan.packard@tch.harvard.edu

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164. THERMODYNAMICS OF COPPER(II) BINDING TO BOVINE SERUM ALBUMIN. **Dean Wilcox** and Yi Zhang, Department of Chemistry, 6128 Burke Laboratory, Dartmouth College, Hanover, NH 03755.

New insight about metal metabolism and metal toxicity will require a better understanding of the interaction of metal ions with proteins, and we have begun studies to quantify the thermodynamics of these interactions. Isothermal titration calorimetry (ITC) has been used to measure metal binding to the protein bovine serum albumin (BSA) and to a three-residue peptide corresponding to the N-terminal BSA sequence, which is the primary Cu(II) binding site. We find that the Cu(II) affinity of the BSA N-terminal site is modulated by the properties of cysteine-34. When this residue is oxidized or chemically derivatized the Cu(II) affinity decreases by nearly an order of magnitude, and this decreased affinity is predominantly due to entropic factors (greater loss of entropy upon Cu(II) binding). We also find that a second Cu(II) binds to Cys-34 of BSA at pH 9.2, and the coordination of the Cu(II) bound at this site has been characterized by UV-vis, CD and EPR spectroscopy. Finally, the temperature dependence of the enthalpy change (ΔH) upon Cu(II) binding to BSA and to the N-terminal tri-peptide has been measured to quantify the contribution of solvent interactions to the thermodynamics of metal binding to this protein.

165. SYNTHESIS AND IN VITRO STUDY OF ^{64}Cu-LABELED DIIMINODIOXIME COMPLEXES FOR EVALUATION OF MULTIDRUG RESISTANCE (MDR). **S. Kiani** and A. Packard, Children's Hospital, Harvard Medical School, Division of Nuclear Medicine, 300 Longwood Avenue, Boston, MA 02115.

The development of multidrug resistance (MDR) is a major problem in chemotherapy. The objective of this project is the development of a lipophilic cationic copper-based PET compound for evaluation of MDR in cancer. As part of the optimization of this agent, we are investigating the effect of the variations in alkyl substituents on the uptake of ^{64}Cu complexes of diiminidioxime ligands by wild-type and drug resistant tumor cells. We have developed a rapid synthesis (<5 min.) for use with high specific-activity ^{64}Cu that produces these complexes in >90% yield and >90% radiochemical purity. The stability of these complexes was tested by equilibration of the ^{64}Cu-complex with human serum/PBS. Accumulation of the ^{64}Cu complexes in the human uterine carcinoma cell line MES-SA and its doxorubicin resistant DX5 derivative was measured. Addition of MDR reversal agent, cyclosporin A did not substantially affect uptake of the ^{64}Cu complexes in MES-SA cells but increased the accumulation in resistant cells to approximately the same value as the parental cells. This difference increases as the lipophilicity of the complexes increases. These results suggest that ^{64}Cu-diiminodioxime complexes are promising agents for PET evaluation of MDR.

DEVELOPMENT OF A ^{64}Cu-BASED PET RADIOPHARMACEUTICAL FOR IMAGING MDR

Alan B. Packard, Ph.D., Salma Kiani, Ph.D., Robert Borgesi, and Eva Barbarics, Ph.D.

Children's Hospital/Harvard Medical School

alan.packard@tch.harvard.edu

Multidrug resistance (MDR) is a treatment-limiting phenomenon frequently encountered during chemotherapy in which a malignancy fails to respond to or becomes resistant to a specific class of chemotherapeutics that includes many of the chemotherapeutic agent that are most effective against breast cancer (e.g., doxorubicin, daunorubicin, paclitaxel). It is characterized by increased concentrations of P-glycoprotein (Pgp) and multidrug-resistance protein (MRP1), which reduce the concentration of these chemotherapeutic agents in the cancer cells. Although well characterized in the laboratory, it is difficult to evaluate multidrug resistance in patients. If this information was available, it could be used to optimize treatment protocols, monitor the development of acquired resistance, and evaluate the effectiveness of MDR modulators.

The objective of this project is to develop a PET (Positron Emission Tomography) radiopharmaceutical for the functional evaluation of MDR. Lipophilic cationic ^{99m}Tc radiopharmaceuticals are known to be substrates for Pgp and MRP1. On this basis, we are developing a lipophilic cationic ^{64}Cu radiopharmaceutical that is a substrate for Pgp and/or MRP1, which can be used for functional imaging of MDR with PET. The basis for the development of this agent is copper complexes of a class of ligands known as diiminedioximes, which can be readily modified to optimize their biological properties.

The uptake of the prototype ^{64}Cu complex by parental and drug resistant MAT-B (rat) and MCF-7 (human) breast cancer cells was measured by incubating a solution of the complex with an equal volume of cell suspension at 37°C, removing samples from the suspension at selected time intervals, and measuring the activity in the cells and the media. The ability of the ^{64}Cu complexes to monitor Pgp expression in the cell lines was measured by repeating this procedure with 10 μM Cyclosporin A added to the incubation media.

The maximum uptake of the ^{64}Cu complex by both cell lines was 1.5% and still increasing at 120 min. At 120 min., uptake of ^{64}Cu by the parental MCF-7 cells is more than twice that of resistant cells. For MAT-B cells, the parental/resistant uptake ratio is approx. 8. In both cases addition of Cyclosporin A increased the uptake: for MAT-B cells to approx. one-half that of the parental cells, for MCF-7 to the same as that of the parental cells. Cyclosporin A had little or no effect on uptake by either parental cell line. For both cell lines, the maximum uptake was approx. 1/3 of that observed for ^{99m}Tc-MIBI.

These results demonstrate that this ^{64}Cu complex is taken up by breast cancer cells and is a substrate for Pgp, but that the biological properties are less than optimal. This optimization is the current objective of this project.

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